Original Article

Vitamins E and C Prevent DNA Double-strand Breaks in Peripheral Lymphocytes Exposed to Radiations from Iodine-131

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Abstract

Purpose: Iodine-131 is used as a radiopharmaceutical to treat thyroid cancer. The current study aimed to evaluate the effects of vitamins E and C on the level of DNA double-strand breaks (DSBs) caused by Radioiodine-131 (I-131) in human lymphocytes. **Materials and Methods:** Whole blood samples from human volunteers were incubated with a certain concentration of vitamins. After 1-h incubation, the samples were incubated with 20 μCi I-131/2 mL (blood + NaCl) for 1 h. To evaluate the effects of antioxidants, lymphocytes were separated, and the mean DSBs/cell was measured for each sample through γ-H2AX assay. **Results:** After 1-h incubation with 20 μCi I-131/2 mL (blood + NaCl), iodine-131 increased the level of DSBs by 102.9%, compared with the background group. Vitamins E and C reduced the level of DSBs by 21.5% and 36.4%, respectively. **Conclusion:** Using vitamins E and C as antioxidants can reduce the toxicity of I-131. Furthermore, vitamin C provided the more protection for DNA, compared with vitamin E.

Keywords: *Double-strand break, iodine-131, radioiodine, Vitamin C, Vitamin E, γ-H2AX foci*

Introduction

In nuclear medicine, iodine-131 is used as a radiopharmaceutical to treat differentiated thyroid cancer.[1,2] Radioiodine-131 (I-131) is considered because of its β-radiation. The particles are main agents to kill cancer cells since β-particles deposit their energy in short-path length; however, damage to healthy cells, as one of its disadvantages, is ineluctable.[1,3,4]

On the other hand, iodine-131 accumulations in nontarget organs and its induced oxidative stress in the patients under treatment results in complications such as xerostomia, dry eye, neck pain, and bone marrow suppression.[5,6] Furthermore, there is a concern for the induction of secondary cancers such as leukemia, stomach, bladder, kidney, and mouth; the increased risk for such secondary cancers in the patients undergoing I-131 therapy has been reported in some studies.[7-9]

Lack of repair or error in repairing double-strand breaks (DSBs) caused by radiation-induced free radicals is one of the factors to increase the cell death and malignancy.[10,11] γ-H2AX assay is one of the methods employed to diagnose DSB, recently used extensively as an efficient method.[12] In this method, the phosphorylated H2AX histone is stained by its specific antibody (called γ-H2AX foci), and then visually counted by the fluorescence microscope; the number of γ-H2AX foci indicates the number of DSB.[12,13] γ-H2AX foci is considered as a genetic damage caused by iodine-131 in patients with thyroid cancer undergoing I-131 therapy.[14-16]

To reduce damages caused by ionizing radiations and protect DNA, radiation protective materials are used;[17,18] vitamins E and C, which have antioxidant properties, are some of these materials.[19,20] According to the conducted studies, vitamin E and lemon candy (contain 3 g of vitamin C) protect salivary glands against I-131 in the patients with different thyroid cancers.[21,22] Furthermore, a combination of vitamins E and C, and selenium reduces oxidative stress in the patients with thyroid cancer undergoing I-131 therapy.[23]

To the best of our knowledge, no study has evaluated the effects of vitamins E and C on the level of DSBs caused by I-131. The current *in vitro* study aimed to evaluate the effects of the mentioned vitamins on the toxicity of iodine-131 by γ-H2AX assay.

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Materials and Methods

Chemical compounds

Iodine-131 was provided by Nuclear Medicine Center of Shahid Beheshti Hospital, Kashan, Iran; Anti-phospho-Histone H2AX was purchased from Millipore company (Millipore, Germany, clone JBW301); Alexa fluor 488 antibody from Cell Signal Technology Inc., and vitamins E and C ampules from Osve Company (Osve Pharmaceutical Company, Tehran, Iran).

Sampling

The study protocol was approved by the Ethical Committee of Kashan University of Medical Sciences (code number: IR. KAUMS. REC.1395.81), and the written consent forms were signed by the participants. The inclusion criteria were being healthy, nonsmoker, and no history of undergoing medical irradiation or chemotherapy at least 2 weeks before the sampling. There were 5 participants (4 males and 1 female) in the current study, with the mean age of 33.6 ± 6.1-year-old (range, 26–42) and the mean weight of 79.4 ± 16.7 kg (range, 57–100). A volume of 6-mL blood sample was taken from each participant, and finally, the samples were equally divided into 6 vials containing ethylenediaminetetraacetic acid (EDTA).

Antioxidants

The effects of vitamins E and C on the level of DSBs caused by I-131 were evaluated. To measure the certain concentration of such vitamins, it was assumed that every person (with the mean weight of 75 kg) totally has 6 L blood. Brand *et al*. protocol was used as the standard concentration (SC) of antioxidants.[24] The employed concentrations are shown in Table 1.

Study design

The current *in vitro* experimental study was designed based on incomplete factorial method, without interaction. The cases were assigned into 2 groups of "with I-131" and "without I-131." The group without I-131 was classified into 3 subgroups: background, vitamin E, and vitamin C. The group with I-131 was also categorized into 3 groups of I-131, I-131+vitamin E, and I-131+vitamin C.

Table 1: The standard concentration of antioxidants added to blood samples

Antioxidants	Vitamin E (ampoule 100 IU/1 mL)	Vitamin C (ampoule 500 mg/5 mL)
SC per 1 mL blood*	0.0666 mg	0.0167 mg
SC per 6 L blood	400 mg	100 mg
RDI/PI per day	10-15 mg/400 mg	100-150 mg

*Concentration of vitamin used in the experiments per 1 mL blood. RDI: Recommended daily intake, PI: Recommendation on package insert, SC: Standard concentration

Treatment and irradiation

The subgroups without I-131 (background: received nothing, antioxidants: a certain concentration of vitamins [Table 1]) were incubated at 37°C for 2 h. Other subgroups, except I-131, were incubated at 37ºC for 1 h with a certain concentration of vitamins [Table 1]; then, 20 μCi I-131 and normal saline, in a total volume of 1 mL, were added to blood samples and reincubated for 1 h. The I-131 subgroup was first incubated at 37°C for 1 h without adding any substance; then, 20 μCi I-131 and normal saline, in a total volume of 1 mL, were added to blood samples and reincubated at 37°C for 1 h. After incubation, the groups were ready for γ-H2AX assay.

γ-H2AX assay

The methodology employed in the current study was based on the protocol described in the previous studies with slight modifications.[14,15,25] The samples containing I-131 were centrifuged at 1500 g for 8 min and the upper solutions (less dense) were discarded. Then, the sediments were washed twice in phosphate-buffered saline (PBS) through centrifugation at 1500 g for 8 min. All blood samples were diluted with PBS (1:1), and the lymphocytes were separated using Ficoll-Paque density gradient, with centrifugation at 1700 g for 15 min at room temperature. The cells were washed twice with PBS, each for 5 min, and fixed with PBS, 4% paraformaldehyde for 15 min. After washing twice with PBS, each for 5 min, the lymphocytes were duplicated and pipetted on slide and left to be dried. The cells were fixed in 100% acetone at −20°C for 10 min; washed three times with PBS and blocked in PBS, 5% bovine serum albumin (BSA) (Sigma Co.), and 0.2% triton x-100 at room temperature. The cells were incubated with the specific γ-H2AX antibody (dilution 1:500) at 4°C overnight. The samples were washed three times, each for 10 min, and stained with the secondary antibody (Alexa Fluor 488) (dilution 1:500) for 1 h at room temperature in the darkness. Then, the cells were washed three times with PBS, each for 15 min, and mounted with propidium iodide (dilution 1:50) (Invitrogen Co.). Finally, in each sample, the number of γ-H2AX foci (DSBs) was visually counted over 100 lymphocyte cells using fluorescence microscope (Ceti, UK) by 2 blind observers. Granulocytes and monocytes were omitted by morphological criteria and the average number of DSB/cell was calculated.

Statistical analysis

First, the normality of data was assessed using Kolmogorov–Smirnov test. Then, the mean and standard deviation (SD) of DSBs were measured in each group. Furthermore, increasing the level of DSBs was calculated using the Formula 1:

$$\frac{\text{with I} - 131 - \text{without I} - 131}{\text{without I} - 131} \times 100$$

The decreased level of DSBs was also calculated using Formula 2:

$$\frac{\text{I}-131-(\text{I}-131+\text{vitamin E or C})}{\text{I}-131}\times 100$$

To compare the groups/subgroups, independent *t*-test, and one-way ANOVA were used. Finally, to evaluate multivariate effects, a generalized linear model (GLM) was used.

Results

Figure 1 shows the γ-H2AX foci in the lymphocytes of the study groups (without I-131 and with I-131). The number of DSBs/cell in the background (without I-131 and antioxidants), vitamin E, and vitamin C groups were 0.126–0.216 (mean ± SD: 0.169 ± 0.031), 0.134–0.207 (mean ± SD: 0.165 ± 0.025), and 0.142–0.227 (mean ± SD: 0.166 ± 0.026), respectively. There was no significant difference among the 3 subgroups of group without I-131 ($P = 0.943$), [Table 2 and Figure 2].

There was an increase in DSBs in the all subgroups of group with I-131 [Figures 1 and 2, Table 2]. The number of DSBs in the I-131 group was 0.310-0.378 DSB/cell (mean ± SD: 0.343 ± 0.023); the level of DSB/cell in this group increased by 102.9%, compared with the background group. The number of DSBs in the group with I-131+vitamin E was 0.221–0.312 DSB/cell (mean ± SD: 0.269 ± 0.025); as it showed 63.0% increase in level of DSBs, compared with the vitamin E group. The number of DSBs in the group with I-131+vitamin C was 0.191–0.240 DSB/cell (mean ± SD: 0.218 ± 0.024); as it showed 31.3% increase in the level of DSBs, compared with the vitamin C group [Table 2 and Figure 2]. There was a significant difference among the 3 subgroups of group with I-131 ($P < 0.001$), [Table 2 and Figure 2].

The level of DSBs decreased by 21.5% (0.269 vs. 0.343 DSB/cell) and 36.4% (0.218 vs. 0.343 DSB/cell) in the I-131+vitamin E and I-131 + vitamin C subgroups, respectively [Table 2 and Figure 2]. Furthermore, for the multivariate analysis of effects of vitamins E and C, with and without using I-131, a GLM was employed. Results of GLM indicated the effect of different groups, in the presence or absence of I-131, on the level of DSBs. Furthermore, the interactive effect of antioxidants on level of DSBs was evaluated, based on the presence or absence of I-131 ($P < 0.001$).

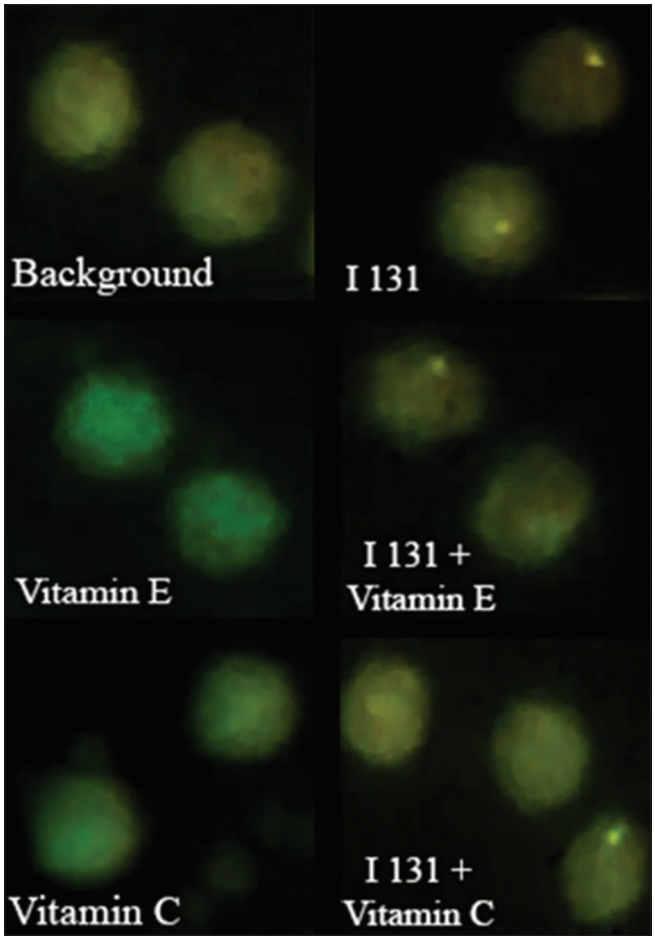

Figure 1: Fluorescence microscopy images of γ-H2AX foci in lymphocytes in the study groups (without I-131 and with I-131). More DNA damage was observed in the group with I-131

Discussion

The effects of vitamins E and C were evaluated on the toxicity of iodine-131 in human lymphocytes in the current *in vitro* study.

In the current study was used 20 μCi I-131/2 mL (blood + NaCl). Since this amount gives 40 mGy absorbed dose to blood, based on the study of Eberlein *et al*.[26] and the current study setups. The dose of 40 mGy is approximately similar to the mean absorbed dose to blood in the patients within the first 2 h of I-131 therapy;[14,27] the highest number of DSBs was observed during this time.[14] Lassmann *et al*.[14] by assessing the number of γ-H2AX foci at different times of receiving iodine-131 in the patients undergoing I-131 therapy, reported that the number of DSBs in the lymphocytes increased and reached its maximum within the first 2 h

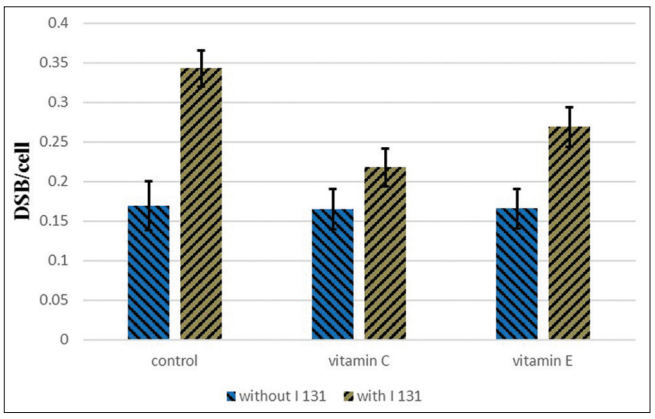

Figure 2: The mean ± standard deviation of double-strand breaks in the study Groups

Table 2: The mean±standard deviation and level of double-strand break changes in the study groups

Groups	DSB/cell (mean±SD)				*P*
	Without I-131	With I-131	Increase of DSB (%)	Decrease of DSB (%)	
Background	0.169±0.031	0.343±0.023	102.9	-	<0.001
Vitamin E	0.165±0.025	0.269±0.025	63.0	21.5	<0.001
Vitamin C	0.166±0.026	0.218±0.024	31.3	36.4	<0.001
P	0.943	<0.001	-	-	-

DSB: Double-strand break, SD: Standard deviation, I-131=Radioiodine-131

of I-131 therapy (median: 0.227 γ-H2AX foci/cell). In the current study, after irradiation of the blood samples with a 40 mGy dose of iodine-131, the number of DSBs reached 0.343 ± 0.023 γ-H2AX foci/cell. The difference between results of these studies may arise from the types of study (*in vivo* vs. *in vitro*) and also different levels of repair in the patients, compared with the blood samples of the current study.

After 1 h incubation with I-131 (without antioxidant), the level of DSBs increased by 102.9%, compared with the background group (0.343 vs. 0.169 DSB/cell). One of the methods to reduce the level of damage caused by ionization radiations and protect from DNA strand is to use the radiation protective materials.[17,18] In this study, vitamin C and vitamin E showed 36.4% and 21.5% decrease in the level of DSBs, respectively ($P < 0.001$). Vitamin C provided the more protection for DNA, compared with vitamin E. According to the study of Brand *et al*.,[24] 1-h preincubation is the golden time for the effectiveness of antioxidants; hence, preincubation with vitamins was set to 1 h. Brand *et al*.[24] evaluated the protective effect of different antioxidants (vitamins E and C, N-acetylcysteine, etc.) on the human lymphocytes by an *in vitro* study. They reported 15% and 25% decrease in the level of DSBs, following the administration of vitamins E and C, respectively. The difference between findings this study and the current study may result from different study methods. In addition to different irradiation and absorbed doses (Isovolt Titan X-ray generator with 10 mGy vs. iodine-131 with 40 mGy), the cases were irradiated only 12 s in the study of Brand *et al*.; while, the current study samples involved antioxidants were incubated with iodine-131 for 1 h. It may result in accumulation effect of antioxidants, and consequently, vitamins showed better performances. However, the protective effect of vitamin C was higher than that of vitamin E in both studies. In another study, Rosário *et al*.[23] evaluated the level of plasma 8-epi-PGF2α (as a marker for lipid peroxidation) in the patients with thyroid cancer undergoing iodine-131 therapy in 2 groups of treatment with antioxidants (a combination of vitamins E and C, and selenium) and treatment without antioxidants. They showed that iodine-131 caused oxidative stress in the patients (112.3% increase in the level of 8-epi-PGF2α after receiving iodine-131) which was much more in the group "treatment without antioxidants," compared with the group "treatment with antioxidants" (112.3% vs. 56.3%). The current *in vitro* study evaluated the level of DSBs in I-131-irradiated human lymphocytes and showed that this radiopharmaceutical significantly increases the number of DSBs (102.9%). In both studies, damages caused by I-131 were minimized by antioxidants. Fallahi *et al*.[21] showed lower levels of salivary glands dysfunction in the patients undergoing I-131 therapy who received vitamin E 800 IU/day for 1 week prior to 4 weeks after the I-131 therapy, compared with the patients in the control group who did not receive vitamin E. The current study also showed that vitamin E can significantly reduce the number of DSBs and protect DNA. According to both studies, vitamin E can significantly reduce DNA damages and complications, such as damages to salivary glands, caused by I-131 therapy.

In the current study, vitamins E and C were only evaluated in the SCs and 1-h preincubation. Different concentrations of vitamins E and C, combination of these vitamins, and also different preincubation time can be employed in the further studies. In addition, further studies can be conducted on other antioxidants and animal models.

Conclusion

The current study indicated that vitamins E and C can reduce damages to DNA caused by iodine-131. According to results of the previous studies[18,21-23] and the current study, these vitamins can preserve DNA and other cellular organelles through scavenging free radicals; consequently, oxidative damage caused by I-131 is minimized, which can reduce complications and improve patients' post-I-131 therapy quality of life.

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Conflicts of interest

There are no conflicts of interest.

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